



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) **EP 0 282 532 B2**

(12) **NEW EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention
of the opposition decision:

30.06.2004 Bulletin 2004/27

(45) Mention of the grant of the patent:

15.12.1993 Bulletin 1993/50

(21) Application number: **87905954.1**

(22) Date of filing: **16.09.1987**

(51) Int Cl.7: **G01N 33/48**

(86) International application number:

PCT/GB1987/000646

(87) International publication number:

WO 1988/002114 (24.03.1988 Gazette 1988/07)

(54) **DETERMINATION OF BIOMASS**

BESTIMMUNG VON BIOMASSE

DETERMINATION DE LA BIOMASSE

(84) Designated Contracting States:

AT BE CH DE FR GB IT LI LU NL SE

(30) Priority: **22.09.1986 GB 8622747**

(43) Date of publication of application:

21.09.1988 Bulletin 1988/38

(73) Proprietor: **ABERTEC LIMITED**

Penglais, Aberystwyth, Dyfed SY23 3DA (GB)

(72) Inventor: **KELL, Douglas Bruce**

Aberystwyth, Dyfed SY23 3HB (GB)

(74) Representative: **Bowles, Sharon Margaret et al**

BOWLES HORTON

Felden House

Dower Mews

High Street

Berkhamsted Hertfordshire HP4 2BL (GB)

(56) References cited:

EP-A- 0 084 874

DE-A- 2 643 871

DE-A- 2 826 517

GB-A- 2 131 954

- **"Determination of biomass concentration by capacitance measurement", Gencer et al. Biotechnology and Bioengineering, Vol. XXI, pp. 1097-1103 (1979)**

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

EP 0 282 532 B2

Description

[0001] This invention relates to a method for the determination of biomass in a culture to a fermentation process in which the method is used as a control feature, and to apparatus for carrying out both the method and the process.

[0002] There is increasing interest in biotechnology, both in the traditional fermentation industry and in the exploitation of living cells in new processes to produce commercially useful products such as antibiotics, vitamins, amino acids and a variety of biologically active proteins.

[0003] The productivity of fermentation processes is dependent to a considerable extent upon culture conditions. It is therefore desirable, and has now become conventional, to control those variables such as pH and dissolved oxygen tension for which sensors are available.

[0004] One of the most important variables in a fermentation process is the reactor biomass concentration, i.e. the concentration of microbial or other cells in the fermenter, since the productivity under a given set of process conditions is directly proportional to this. However, to date no accurate means has been developed for measuring the biomass content of a culture in real time, i.e. for measuring the present biomass content rather than the biomass content some time in the past.

[0005] The lack of suitable means to measure this important process variable has been commented upon by several writers in recent years, see for example: Pirt, "Principles of Microbe and Cell Cultivation", Blackwell, 1975, p 16; Carleysmith and Fox, "Fermenter Instrumentation and Control", Adv. Biotechnol. Processes 3, 1-51, 1984; and Harris and Kell, "The estimation of Microbial Biomass," Biosensors J.1. 17-84 (1985).

[0006] This last reference notes (1) that the most appropriate measure suitable for estimating biomass in real time is the biovolume, i.e. the volume fraction of a culture enclosed by the cytoplasmic membranes of the microbial or other cells within it, (2) that the only means by which biomass might be estimated in real time will be by physical as opposed to chemical measurements, and (3) that all presently available physical methods (such as light scattering) for estimating biomass, are essentially unusable under the difficult conditions existing in a fermenter.

[0007] It is an object of the present invention to provide a measurement of the microbial or other biomass in fermenters in real time and preferably in situ.

[0008] According to the present invention, we provide a method for the determination of biomass in a culture comprising a suspending fluid and cells, the method comprising generating a signal dependent on the dielectric permittivity of material in the bulk of the medium using electrical capacitance measurement, at a predetermined frequency, between electrodes mutually spaced in the medium, said frequency being selected in the half of the β -dispersion which occurs at lower fre-

quencies but a which α -dispersion is substantially insignificant such that the dielectric permittivity varies with the volume fraction of the medium enclosed by the cytoplasmic membranes of the cells, and determining said volume fraction from the permittivity dependent signal.

[0009] Further in accordance with the invention, we also provide a fermentation process utilising a culture comprising a suspending liquid and cells, the process comprising generating a signal dependent on the dielectric permittivity of material in the bulk of the culture using electrical capacitance measurement, at a predetermined frequency, between electrodes mutually spaced in the culture or a sample thereof, and providing an indication if the permittivity dependent signal differs from a predetermined value or falls outside a predetermined range, and/or altering the value of a process parameter to return the signal towards the predetermined value or the predetermined range, said predetermined frequency being selected in the half of the β -dispersion which occurs at lower frequencies but a which α -dispersion is substantially insignificant such that the dielectric permittivity varies with the volume fraction of the culture enclosed by the cytoplasmic membranes of the cells.

[0010] Yet further in accordance with the invention we provide apparatus for performing a fermentation utilising a culture comprising a fermenter containing the culture, electrodes mutually spaced in the fermenter so as to be in contact with the culture; and means for generating a signal dependent on the dielectric permittivity in the bulk of the culture using electrical capacitance measurement between the electrodes, at a predetermined frequency which is selected in the half of the β -dispersion which occurs at lower frequencies but a which α -dispersion is substantially insignificant such that the dielectric permittivity varies with the volume fraction of the medium enclosed by the cytoplasmic membranes of the cells.

[0011] The method is most useful for determining biomass in a culture and will generally be described in relation to such use in this specification. However it may also be used to determine biomass suspended in aqueous and in other suspensions and for instance in emulsions.

[0012] The apparatus may include means to determine whether the capacitance dependent signal differs from a predetermined value or falls outside a predetermined range of values.

[0013] The apparatus may further include means responsive to the means to determine, for altering one or more parameters of the process to return the capacitance dependent signal towards the predetermined value or the predetermined range.

[0014] There is an important relationship between the apparent relative permittivity of a suspension of spherical cells measured at a particular frequency, the cell radius, and the cell volume. The relationship can be expressed by the equation:

$$\epsilon_L = \frac{9, P r C_m}{4 \epsilon_r} + \epsilon_{\infty}$$

where

ϵ_L is the apparent relative permittivity of the culture
 P is the volume fraction of the culture occupied by cells
 r is the cell radius
 C_m is the capacitance of the cell membrane per unit area, and
 ϵ_{∞} is the permittivity of the culture at a frequency which is high with respect to the measuring frequency
 ϵ_r is the permittivity of free space, approximately 8.85×10^{-12} F/m.

[0015] For non-spherical cells, the factor 9/4 is modified.

[0016] Permittivity is related to capacitance, which can be measured, by a factor, referred to as the cell constant, which depends on the electrode number, size and geometry. The permittivity of a sample can thus be determined by measuring the capacitance between electrodes mutually spaced therein. Formerly permittivity was known as dielectric constant.

[0017] The relative permittivity of an aqueous solution is dimensionless and depends slightly on the temperature and electrical conductivity of the sample but is always in the range 60 to 85, very often being approximately 78. The permittivity of aqueous solutions, when measured at low voltages, is independent of frequency up to approximately 1GHz, and under the conditions normally pertaining in a fermenter, is essentially unaffected by the presence of dissolved gases and non-cellular particulate matter.

[0018] The permittivity of an aqueous solution forms a reference or baseline against which the cell content of a culture may be determined.

[0019] The permittivity of a cellular suspension is strongly dependent upon frequency. For example, the permittivity at 100 kHz, of a suspension of bacterial cells of radius 0.5 μ m and occupying a volume fraction of 0.1, might be approximately 250. This value will decrease towards a baseline value corresponding essentially to that of the suspending liquid as the frequency is increased.

[0020] The frequency dependent increase in permittivity found for cell suspensions over the baseline value for an aqueous solution, is known as a dispersion. Three major dispersions (α , β , and γ dispersions) are generally recognised, with the β -dispersion being particularly important for the invention. The α -dispersion occurs at lower frequencies than the β -dispersion and is mainly caused by the presence of mobile ions at the cell surface. The γ -dispersion occurs at higher frequencies than the β -dispersion and is caused predominantly by the ro-

tation of dipolar species such as water. The α -dispersion is highly dependent on the structure of the cell wall, whilst the γ -dispersion is not specific for intact cells.

[0021] The β -dispersion of the dielectric permittivity was named by H P Schwan (Advances in Biological and Medical Physics, Vol. 5, 147-209, 1957). It is caused mainly by the presence of relatively ion-impermeable cellular membranes and has the approximate shape of an inverted sigmoid. Its magnitude for spherical cells, given C_m , can be taken to be dependent only upon cell radius and the volume fraction occupied by the cells. Other influences are, or can be taken to be, constants. Its position on the frequency axis depends upon the cell radius and the internal and external electrical conductivities. The large magnitude of the β -dispersion is due to cells having a conducting interior separated from the exterior by a poorly-conducting membrane providing a large capacitance, for example 1 μ F/cm². Its magnitude is therefore directly proportional to the volume fraction occupied by the cells. The magnitude of the β -dispersion is large relative to the effects of non-cellular particles, dissolved gasses or oil droplets in the suspension. This allows the biovolume (related to the biomass by the density of the cell cytoplasm) to be measured without being significantly affected by particles or oil droplets in cell suspensions. The β -dispersion is a property of intact cells (see Pethig "Dielectric and Electronic Properties of Biological materials", Wiley, 1979) and its magnitude is directly proportional to the volume fraction of cells in a suspension up to very high volume fractions.

[0022] Suitable frequencies for measurement of biomass include frequencies at which the β -dispersion is substantially complete but at which the α -dispersion is substantially insignificant, optimum measuring frequencies are in the half of the β -dispersion which occurs at lower frequencies before the α -dispersion is reached. Suitable frequencies include radio frequencies from 0.1 to 10 MHz, especially 0.1 MHz to 1 MHz, with a preferred range being 0.2 to 0.3 MHz. For large cells frequencies below 0.1 MHz may be found suitable, whilst for cultures having a high electrical conductivity, frequencies in excess of 1 MHz may be found suitable.

[0023] The method of the invention can be used to determine the biomass content of a wide range of cultures, including plant, animal and microbial cell cultures, and the fermentation process can be used to produce a wide variety of products. Most usefully, however, the method of the invention is applicable to microbial cultures containing bacterial, yeast or fungal cells. Frequently the suspending liquid will be an aqueous culture medium containing nutrients suitable for the cells suspended in it, for example compounds containing carbon, phosphorus, nitrogen and other sources of essential nutrients.

[0024] The fermentation process of the invention can be carried out in any type of fermenter. Permittivity can be determined by measuring the capacitance between electrodes mutually spaced in a sample of the culture. This can be done using a bridge or other circuit. Prefer-

ably the measuring electrodes are attached to the fermenter used in the process of the invention to enable direct on-line measurements to be made. Alternatively, samples may be withdrawn from the fermenter.

[0025] Examples of the method and process, and an embodiment of the apparatus of the invention, will now be described with reference to the accompanying drawings, in which:

Figure 1 shows typical variations of capacitance against frequency at different concentrations of cells in a culture;

Figure 2 shows the variation with time of capacitance, at fixed frequency, as a culture grows in a gas-lift fermenter;

Figure 3 shows the capacitance plotted against optical density of samples withdrawn from the fermenter; and

Figure 4 is a schematic diagram of apparatus embodying the invention.

[0026] Referring to Figure 4, a gas-lift fermenter 2 has a pair of spaced wire electrodes 4 fitted in the base. The electrodes 4 are connected to respective terminals of a Hewlett Packard low frequency impedance analyser 6 type No. 4192A. In order to reduce the effects of the capacitance inherent in the connecting leads, the impedance analyser 6 is placed as close as possible to the connections with the electrodes. Indeed, the impedance analyser may be beneath the fermenter. In order to reduce the effects of polarisation of the culture medium at the electrodes, these are platinum black. Air is supplied to the culture via an inlet 8.

[0027] *Saccharomyces cerevisiae* is grown in the gas-lift fermenter. The culture medium contains 5% (w/v) malt extract and 0.5% (w/v) yeast extract. The initial pH is adjusted to 4.5 and the temperature is 30 degrees centigrade. The conductance of this medium is ca. 1.57 mS/cm. Typical frequency dependent capacitance is shown in Figure 1 at a number of different cell concentrations. It is evident that there is a sizable dielectric dispersion which depends on cell concentration. The dispersion corresponds in magnitude and relaxation time to the β -dispersion. At a fixed frequency of 0.3 MHz, Figures 2 and 3 show respectively, the change in capacitance with time and against the optical density of samples taken from the fermenter and appropriately diluted.

[0028] The productivity of a fermentation process is dependent on culture conditions. One of the important variables in the fermentation process which it is advantageous to measure, is the biomass concentration. It may also be advantageous to run the fermentation at a particular biomass concentration, or with the concentration within a predetermined range.

[0029] The apparatus described above can be calibrated to determine the capacitance at a fixed frequency, say 0.3 MHz, corresponding to particular biomass concentrations. The apparatus may be calibrated to in-

dicating biomass directly.

[0030] The on line measurement of capacitance, or biomass, provides a capacitance dependent signal which is fed to a process controller 10 where it is compared with a reference signal. Alternatively the process controller may determine whether the capacitance dependent signal lies within a predetermined range. The process controller is arranged to adjust one or more parameters of the fermentation process to return the capacitance dependent signal towards the reference signal value or towards the predetermined range. Parameters which may be adjusted include the oxygen rate, i.e. the air supply rate, the dilution rate, the rate of addition of nutrients or new culture in a continuous process, the temperature, and so on.

[0031] A preferred specific embodiment of an apparatus for use in the invention is described in our copending UK Application no. 8622748 (corresponding to EP-02 816 02 identified by the reference B 34038 filed by inventors Kell and Todd on the same day as this application), the contents of which are incorporated herein by reference.

Claims

1. A method for the determination of biomass in a culture comprising a suspending fluid and cells, the method comprising generating a signal dependent on the dielectric permittivity of material in the bulk of the medium using electrical capacitance measurement, at a predetermined frequency, between electrodes (4) mutually spaced in the medium, said frequency being selected in the half of the β -dispersion which occurs at lower frequencies but a which α -dispersion is substantially insignificant such that the dielectric permittivity varies with the volume fraction of the medium enclosed by the cytoplasmic membranes of the cells, and determining said volume fraction from the permittivity dependent signal.
2. A fermentation process utilising a culture comprising a suspending liquid and cells, the process comprising generating a signal dependent on the dielectric permittivity of material in the bulk of the culture using electrical capacitance measurement, at a predetermined frequency, between electrodes (4) mutually spaced in the culture or a sample thereof, and providing an indication if the permittivity dependent signal differs from a predetermined value or falls outside a predetermined range, and/or altering the value of a process parameter to return the signal towards the predetermined value or the predetermined range, said predetermined frequency being selected in the half of the β -dispersion which occurs at lower frequencies but a which α -dispersion is substantially insignificant such that the dielectric permittivity varies with the volume fraction of the

culture enclosed by the cytoplasmic membranes of the cells.

3. Apparatus for performing a fermentation utilising a culture comprising a suspending liquid and cells, the apparatus comprising a fermenter (2) containing the culture, electrodes (4) mutually spaced in the fermenter (2) so as to be in contact with the culture; and means (6) for generating a signal dependent on the dielectric permittivity in the bulk of the culture using electrical capacitance measurement between the electrodes (4), at a predetermined frequency which is selected in the half of the β -dispersion which occurs at lower frequencies but a which α -dispersion is substantially insignificant such that the dielectric permittivity varies with the volume fraction of the medium enclosed by the cytoplasmic membranes of the cells.
4. Apparatus as claimed in claim 3, including means (10) to determine whether the capacitance dependent signal differs from a predetermined value or falls outside a predetermined range of values.
5. Apparatus as claimed in claim 4, including means responsive to the means to determine, for altering one or more parameters of the process to return the capacitance dependent signal towards the predetermined value or the predetermined range.
6. Apparatus as claimed in any of claims 3 to 5, wherein the predetermined frequency is in the range 0.1mHz to 10MHz.

Patentansprüche

1. Verfahren zum Bestimmen von Biomasse in einer Kultur die Suspensionsfluid und Zellen enthält, wobei ein von der Dielektrizitätskonstante von Material im Inneren des Mediums abhängiges Signal erzeugt wird, indem vom Messen der elektrischen Kapazität zwischen voneinander beabstandeten Elektroden (4) in dem Medium bei einer vorbestimmten Frequenz Gebrauch gemacht wird, die in der Hälfte der β -Dispersion gewählt wird, welche bei niedrigeren Frequenzen auftritt, aber bei der α -Dispersion im Wesentlichen unbedeutend ist, so dass die Dielektrizitätskonstante sich mit dem durch die Zellplasmamembrane der Zellen eingeschlossenen Volumenanteil des Mediums ändert, und der Volumenanteil aus dem von der Dielektrizitätskonstante abhängiges Signal bestimmt wird.
2. Fermentierprozess, bei dem eine Kultur benutzt wird, die eine Suspensionsflüssigkeit und Zellen enthält, wobei ein von der Dielektrizitätskonstante von Material im Inneren der Kultur abhängiges Si-

gnal erzeugt wird, indem vom Messen der elektrischen Kapazität zwischen voneinander beabstandeten Elektroden (4) in der Kultur oder in einer Probe hiervon bei einer vorbestimmten Frequenz Gebrauch gemacht wird, und eine Anzeige abgegeben wird, falls das von der Dielektrizitätskonstante abhängige Signal von einem vorbestimmten Wert abweicht oder aus einem vorbestimmten Bereich fällt, und/oder der Wert eines Prozessparameters für das Zurückführen des Signals zu dem vorbestimmten Wert oder zu dem vorbestimmten Bereich geändert wird, wobei die vorbestimmte Frequenz in der Hälfte der β -Dispersion gewählt wird, welche bei niedrigeren Frequenzen auftritt, aber bei der α -Dispersion im Wesentlichen unbedeutend ist, so dass die Dielektrizitätskonstante sich mit dem durch die Zellplasmamembrane der Zellen eingeschlossenen Volumenanteil der Kultur ändert.

3. Gerät für das Ausführen einer Fermentation unter Benutzung einer Kultur, die eine Suspensionsflüssigkeit und Zellen enthält, wobei das Gerät ein Fermentiergefäß(2) zur Aufnahme der Kultur, Elektroden (4), die in dem Fermentiergefäß (2) in gegenseitigem Abstand derart angeordnet sind, dass sie mit der Kultur in Kontakt sind, und eine Einrichtung (6) zum Erzeugen eines Signals enthält, das von der Dielektrizitätskonstante im Inneren der Kultur abhängt, indem vom Messen der elektrischen Kapazität zwischen den Elektroden (4) bei einer vorbestimmten Frequenz Gebrauch gemacht wird, die in der Hälfte der β -Dispersion gewählt ist, welche bei niedrigeren Frequenzen auftritt, aber bei der α -Dispersion im Wesentlichen unbedeutend ist, so dass die Dielektrizitätskonstante sich mit dem durch die Zellplasmamembrane der Zellen eingeschlossenen Volumenanteil des Mediums ändert.
4. Gerät nach Anspruch 3, das eine Einrichtung (10) enthält, die bestimmt, ob das von der Kapazität abhängige Signal sich von einem vorbestimmten Wert unterscheidet oder aus einem vorbestimmten Bereich von Werten herausfällt.
5. Gerät nach Anspruch 4, das eine auf die Bestimmung. einrichtung ansprechende Einrichtung enthält, die einen oder mehrere Prozessparameter ändert, um das von der Kapazität abhängige Signal zu dem vorbestimmten Wert oder dem vorbestimmten Bereich hin zu ändern.
6. Gerät nach einem der Ansprüche 3 bis 5, wobei die vorbestimmte Frequenz in einem Bereich von 0,1 MHz bis 10 MHz liegt.

Revendications

1. Procédé pour déterminer la biomasse dans une culture comprenant un fluide de suspension et des cellules, le procédé consistant à produire un signal dépendant de la permittivité diélectrique du matériau dans la masse du milieu moyennant l'utilisation d'une mesure de capacité électrique, à une fréquence prédéterminée, entre des électrodes (4) mutuellement espacées dans le milieu, ladite fréquence étant choisie dans la moitié de la dispersion β qui se produit aux fréquences inférieures mais où la dispersion α est essentiellement négligeable, de telle sorte que la permittivité diélectrique varie avec la fraction volumique du milieu qui est renfermé entre les membranes cytoplasmiques des cellules, et à déterminer ladite fraction volumique à partir du signal qui dépend de la permittivité. 5

2. Procédé de fermentation utilisant une culture comprenant un liquide de suspension et des cellules, le procédé consistant à produire un signal dépendant de la permittivité diélectrique du matériau dans la masse de la culture en utilisant une mesure de capacité électrique, à une fréquence prédéterminée, entre des électrodes (4) réciproquement espacées dans la culture ou un échantillon de cette dernière, et à délivrer une indication dans le cas où le signal, qui dépend de la permittivité, diffère d'une valeur prédéterminée ou tombe à l'extérieur d'une gamme prédéterminée et/ou modifier la valeur d'un paramètre du processus pour ramener, le signal vers la valeur prédéterminée ou la gamme prédéterminée, ladite fréquence prédéterminée étant choisie dans la moitié de la dispersion β qui se produit aux fréquences inférieures mais où la dispersion α est essentiellement négligeable, de telle sorte que la permittivité diélectrique varie avec la fraction volumique de la culture renfermée entre les membranes cytoplasmiques des cellules. 20 25 30 35 40

3. Dispositif pour exécuter une fermentation moyennant l'utilisation d'une culture comprenant un liquide de suspension et des cellules, le dispositif comportant un appareil de fermentation (2) contenant la culture, des électrodes (4) disposées réciproquement dans le dispositif de fermentation (2) de manière à être en contact avec la culture ; et des moyens (6) pour produire un signal qui dépend de la permittivité diélectrique dans la masse de la culture, moyennant l'utilisation d'une mesure de capacité électrique entre les électrodes (4) à une fréquence prédéterminée qui est choisie dans la moitié de la dispersion β qui se produit aux fréquences inférieures mais où la dispersion α est essentiellement négligeable, de telle sorte que la permittivité diélectrique varie avec la fraction volumique du milieu renfermé entre les membranes cytoplasmiques 45 50 55

des cellules..

4. Dispositif selon la revendication 3, comprenant des moyens (10) pour déterminer si le signal, qui dépend de la capacité, diffère d'une valeur prédéterminée ou tombe à l'extérieur d'une gamme prédéterminée de valeurs. 5

5. Dispositif selon la revendication 4, comprenant des moyens répondant aux moyens de détermination pour modifier un ou plusieurs paramètres du processus de manière à ramener le signal qui dépend de la capacité vers la valeur prédéterminée ou la gamme prédéterminée. 10 15

6. Dispositif selon l'une quelconque des revendications 3 à 5, dans lequel ladite fréquence prédéterminée se situe dans la gamme allant de 0,1 MHz à 10 MHz. 20

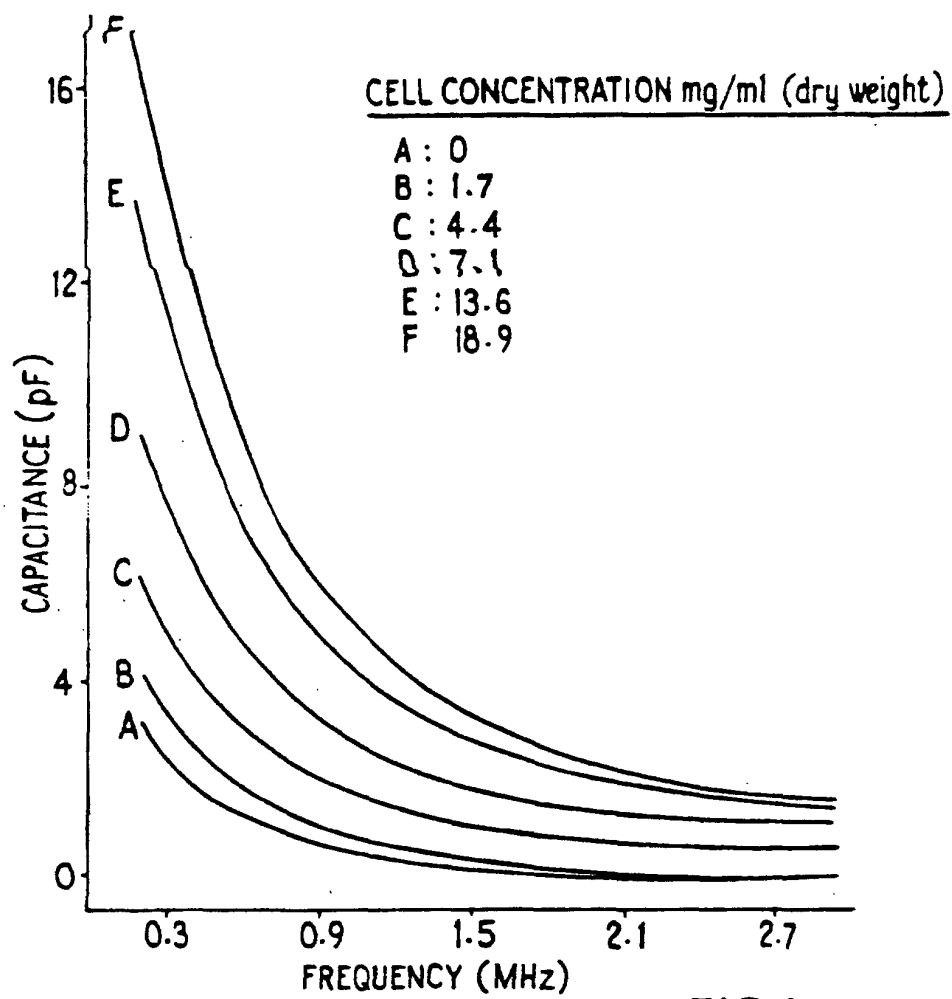


FIG.1

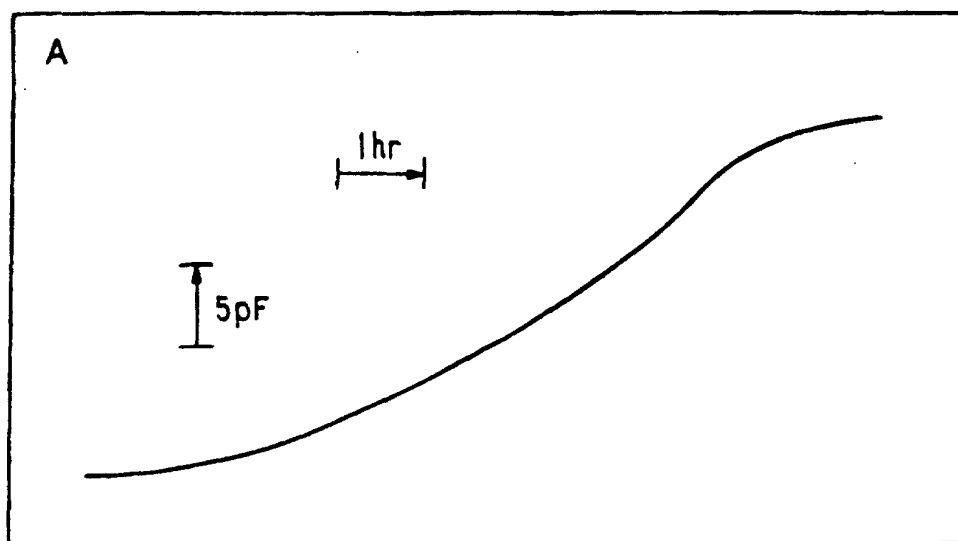


FIG.2

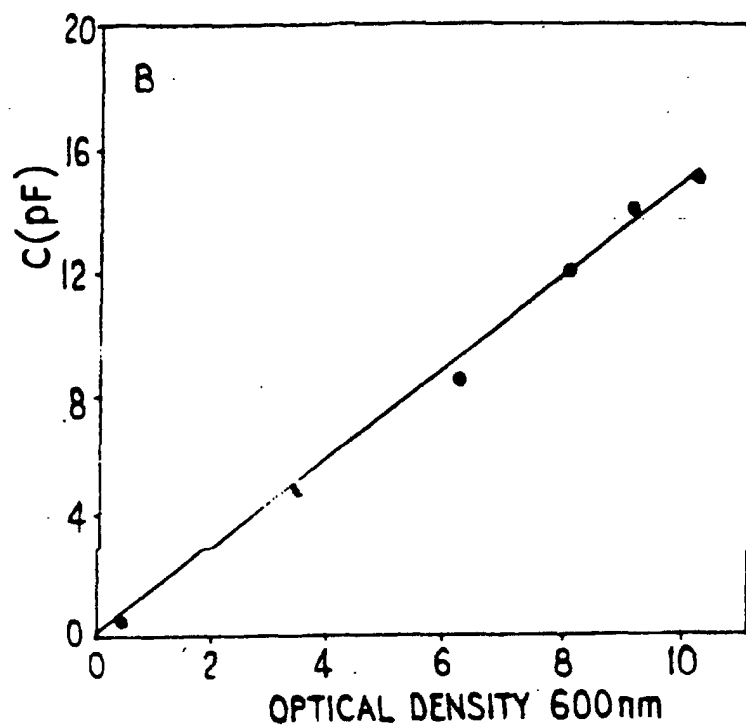


FIG.3

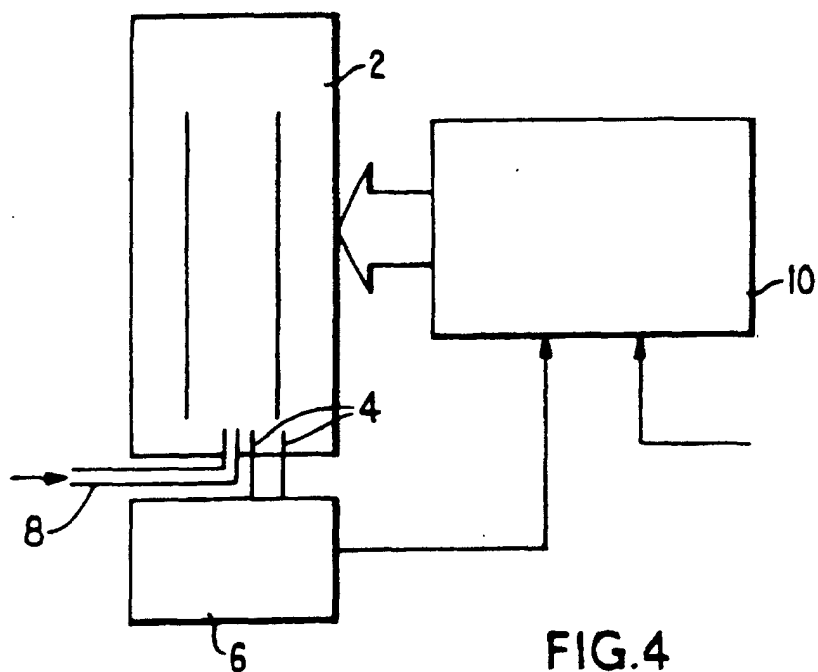


FIG.4